



Sample name: **Pyridoxine HCl**
Assay name: **UV-metric psKa**
Assay ID: **18E-22013**
Filename: **C:\Sirius_T3\18E-22013_Pyridoxine HCl_UV-metric psKa_0417936-0002.t3r**

Experiment start time: **5/22/2018 2:50:50 PM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

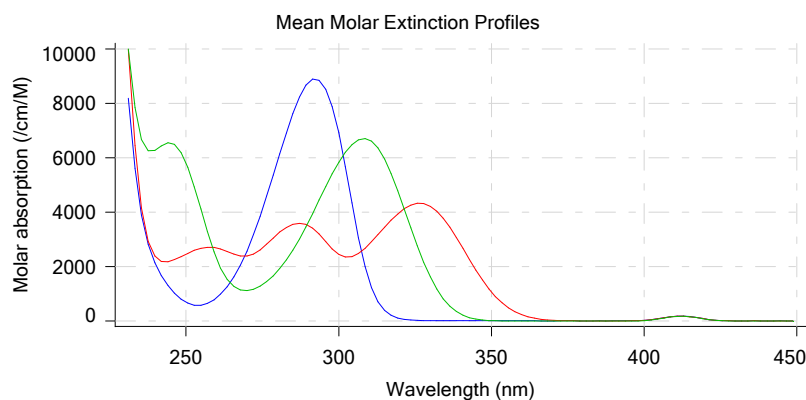
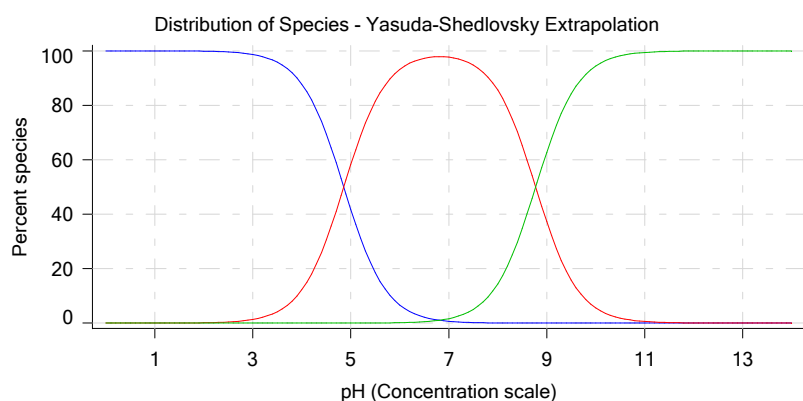
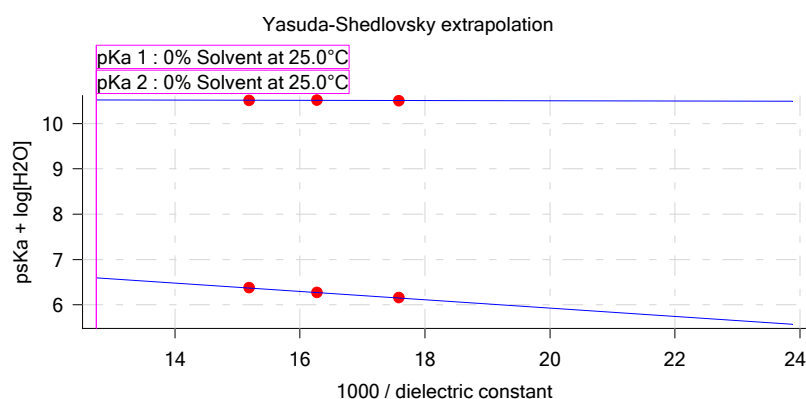
Yasuda-Shedlovsky result

Extrapolation type	pKa 0%	SD	Intercept	Slope	R ²	Ionic strength	Temperature
Yasuda-Shedlovsky	4.86	±0.01	7.77	-91.8929	0.9995	0.167 M	25.0°C
Yasuda-Shedlovsky	8.77	±0.01	10.55	-2.4900	0.3098	0.167 M	25.0°C

Component assay results

Titration	Methanol weight%	Direction	Result type	Dielectric constant	[H2O]	Ionic strength	Temperature	psKa 1	psKa 2
18E-22013 Points 4 to 43	48.94 %	Up	UV-metric pKa	56.9	25.0 M	0.158 M	25.0°C	✓ 4.76	✓ 9.11
18E-22013 Points 45 to 85	38.99 %	Up	UV-metric pKa	61.4	30.6 M	0.168 M	25.0°C	✓ 4.79	✓ 9.03
18E-22013 Points 87 to 120	29.29 %	Up	UV-metric pKa	65.8	36.3 M	0.175 M	25.0°C	✓ 4.82	✓ 8.95

Graphs



UV-metric psKa_0417936-0002 Titration 1 of 3 18E-22013 Points 4 to 43

Results

pKa 1 **4.76**
pKa 2 **9.11**
RMSD **0.037 0.030 0.024**
Chi squared **0.1583**
PCA calculated number of pKas **4**
Average ionic strength **0.158 M**
Average temperature **25.0°C**
Analyte concentration range **60.6 µM to 56.8 µM**
Methanol weight % **48.9 %**
Dielectric constant **56.9**
Water concentration **25.0 M**

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Experiment start time: **5/22/2018 2:50:50 PM**
 Analyst: **Dorothy Levorse**
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Results (continued)

Number of pKas source **Predicted**
 Wavelength clipping **230.0 nm to 450.0 nm**
 pH clipping **1.458 to 12.540**

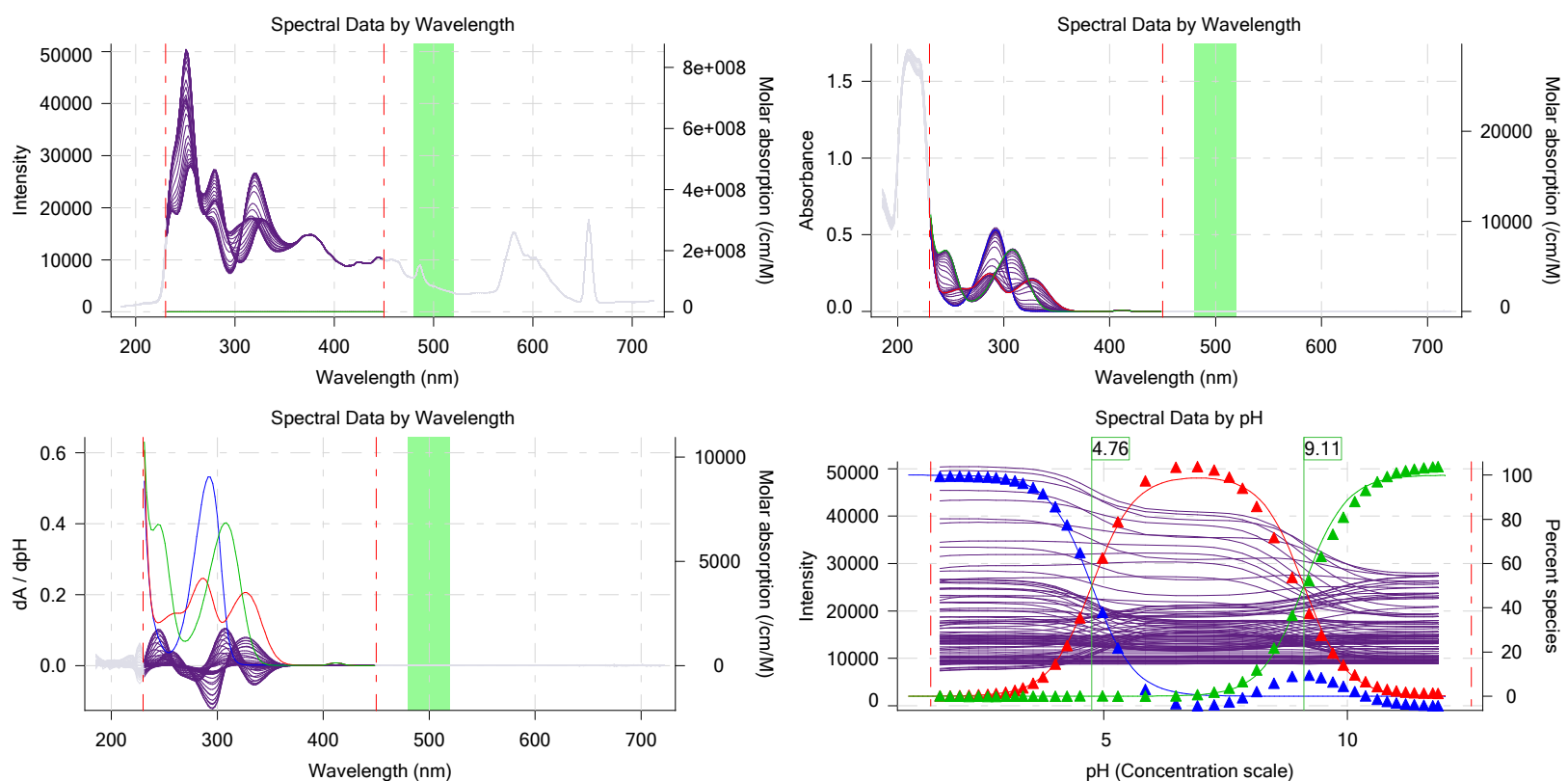
Warnings and errors

Errors None
 Warnings PCA calculation disagrees with predicted number of pKas

Assay Settings

Setting	Value	Original Value	Date/Time changed	Imported from
Buffer in use	Yes			
Buffer type	Phosphate Buffer			
Assay Medium				
Volume of buffer introduced	0.025000 mL			
Add buffer manually	Manual			

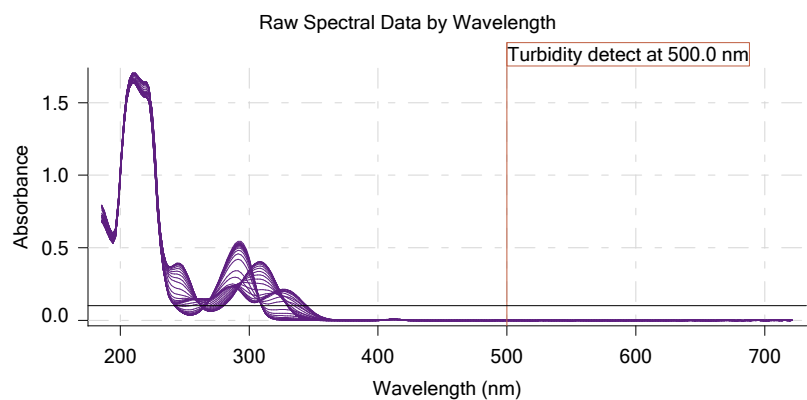
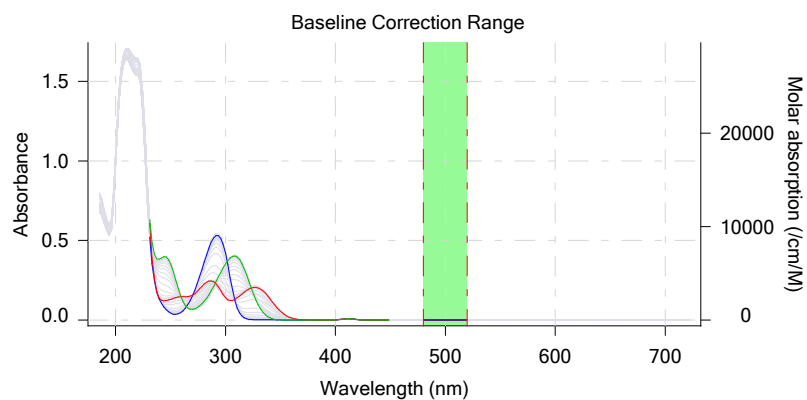
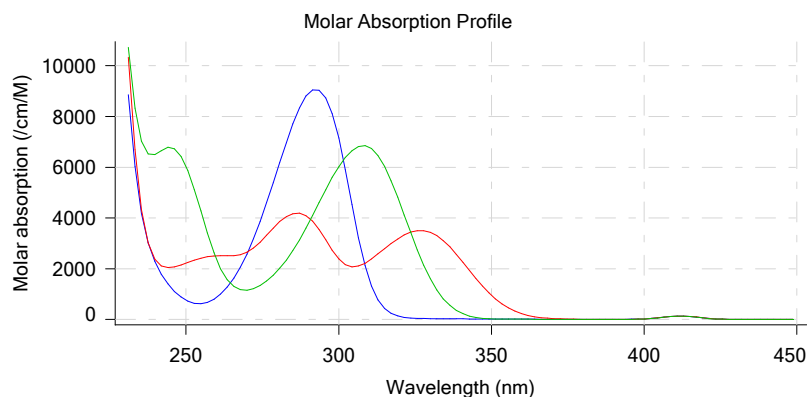
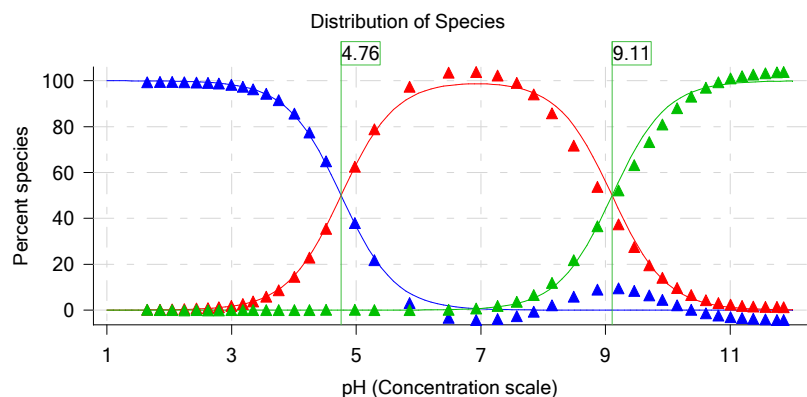
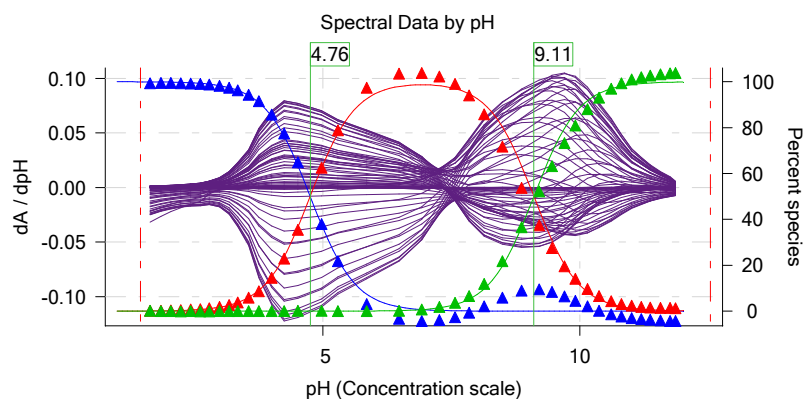
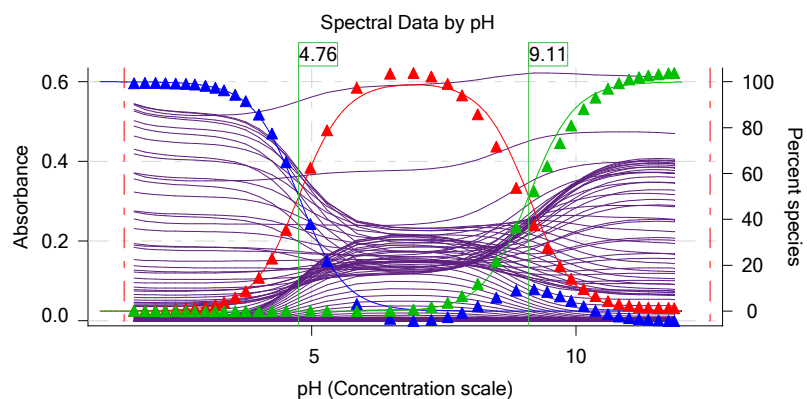
Graphs



Sample name: **Pyridoxine HCl**
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Experiment start time: **5/22/2018 2:50:50 PM**
 Analyst: **Dorothy Levorse**
 Instrument ID: **T311053**

Graphs (continued)



UV-metric psKa_0417936-0002 Titration 2 of 3 18E-22013 Points 45 to 85

Results

pKa 1 **4.79**
 pKa 2 **9.03**
 RMSD **0.041 0.031 0.029**
 Chi squared **0.2196**
 PCA calculated number of pKas **3**
 Average ionic strength **0.168 M**
 Average temperature **25.0°C**
 Analyte concentration range **49.2 µM to 46.2 µM**
 Methanol weight % **39.0 %**
 Dielectric constant **61.4**
 Water concentration **30.6 M**

Sample name: **Pyridoxine HCl**
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Experiment start time: **5/22/2018 2:50:50 PM**
 Analyst: **Dorothy Levorse**
 Instrument ID: **T311053**

Results (continued)

Number of pKas source **Predicted**
 Wavelength clipping **230.0 nm to 450.0 nm**
 pH clipping **1.475 to 12.537**

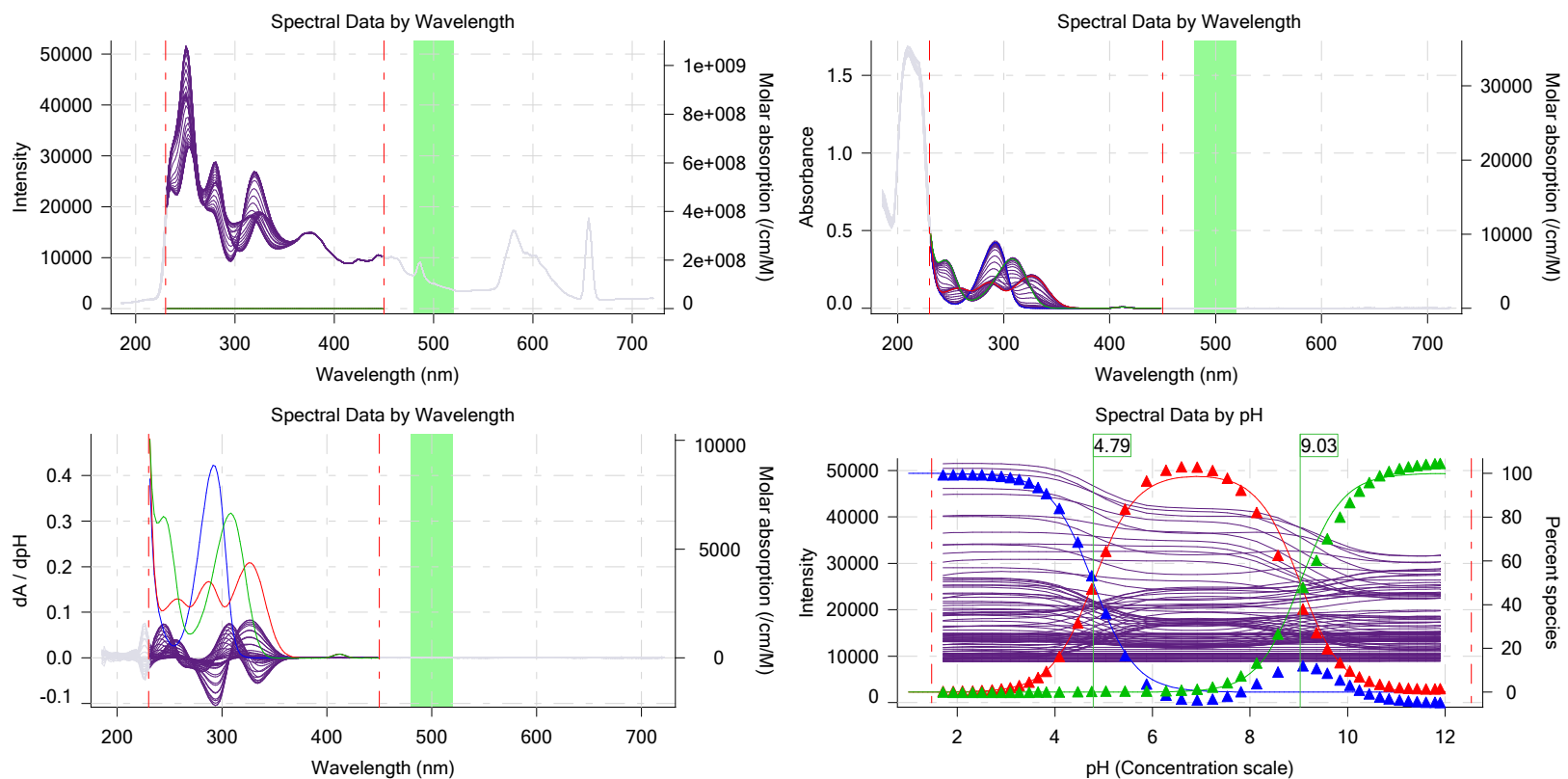
Warnings and errors

Errors **None**
 Warnings **PCA calculation disagrees with predicted number of pKas**

Assay Settings

Setting	Value	Original Value	Date/Time changed	Imported from
Buffer in use	Yes			
Buffer type	Phosphate Buffer			
Assay Medium				
Volume of buffer introduced	0.025000 mL			
Add buffer manually	Manual			

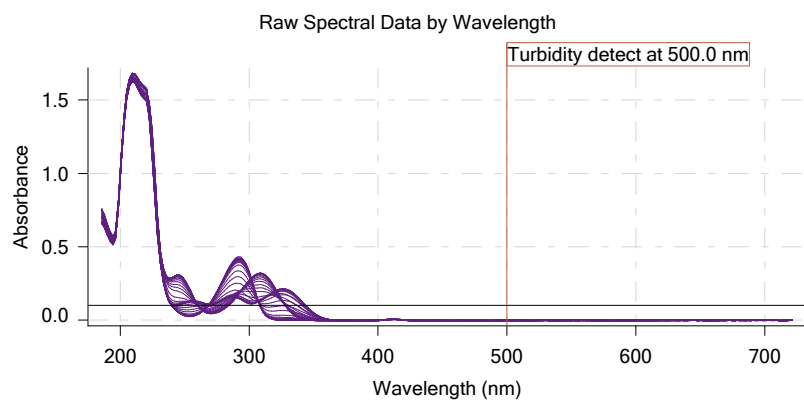
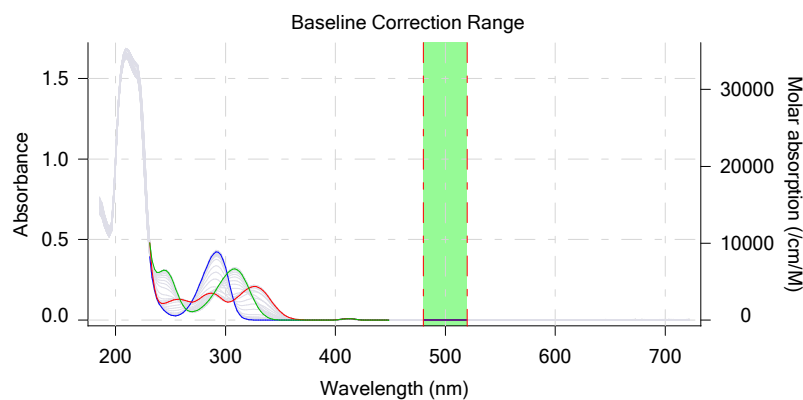
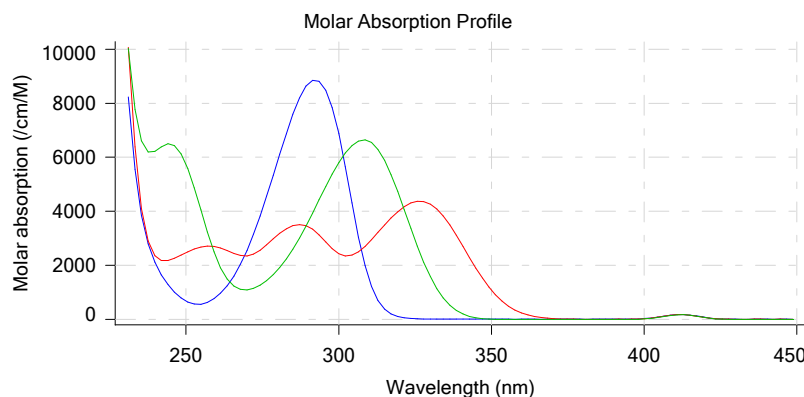
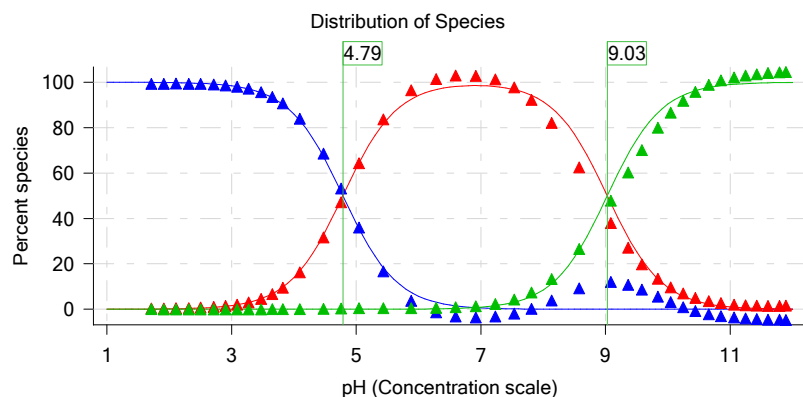
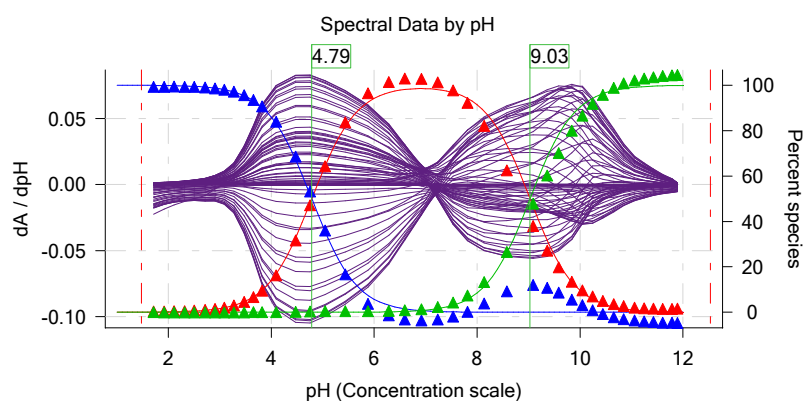
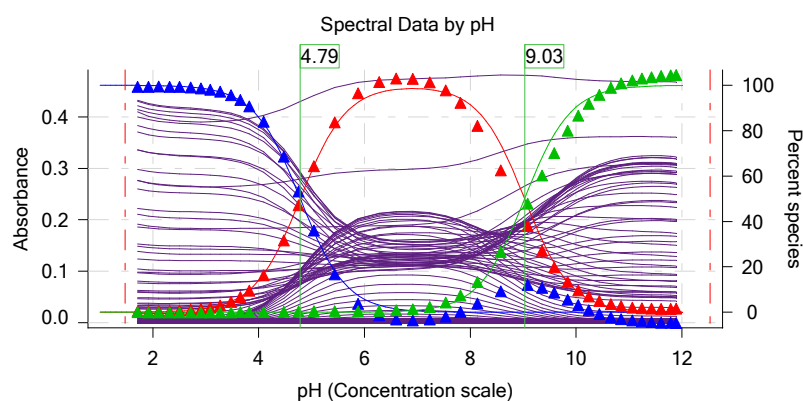
Graphs



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Experiment start time: **5/22/2018 2:50:50 PM**
 Analyst: **Dorothy Levorse**
 Instrument ID: **T311053**

Graphs (continued)



UV-metric psKa_0417936-0002 Titration 3 of 3 18E-22013 Points 87 to 120

Results

pKa 1 **4.82**
 pKa 2 **8.95**
 RMSD **0.040 0.023 0.034**
 Chi squared **0.2385**
 PCA calculated number of pKas **2**
 Average ionic strength **0.175 M**
 Average temperature **25.0°C**
 Analyte concentration range **37.7 µM to 35.4 µM**
 Methanol weight % **29.3 %**
 Dielectric constant **65.8**
 Water concentration **36.3 M**

Sample name: **Pyridoxine HCl**

Assay name: **UV-metric psKa**

Assay ID: **18E-22013**

Filename: **C:\Sirius_T3\18E-22013_Pyridoxine HCl_UV-metric psKa_0417936-0002.t3r**

Experiment start time: **5/22/2018 2:50:50 PM**

Analyst: **Dorothy Levorse**

Instrument ID: **T311053**

Results (continued)

Number of pKas source **Predicted**

Wavelength clipping **230.0 nm to 450.0 nm**

pH clipping **1.497 to 12.523**

Warnings and errors

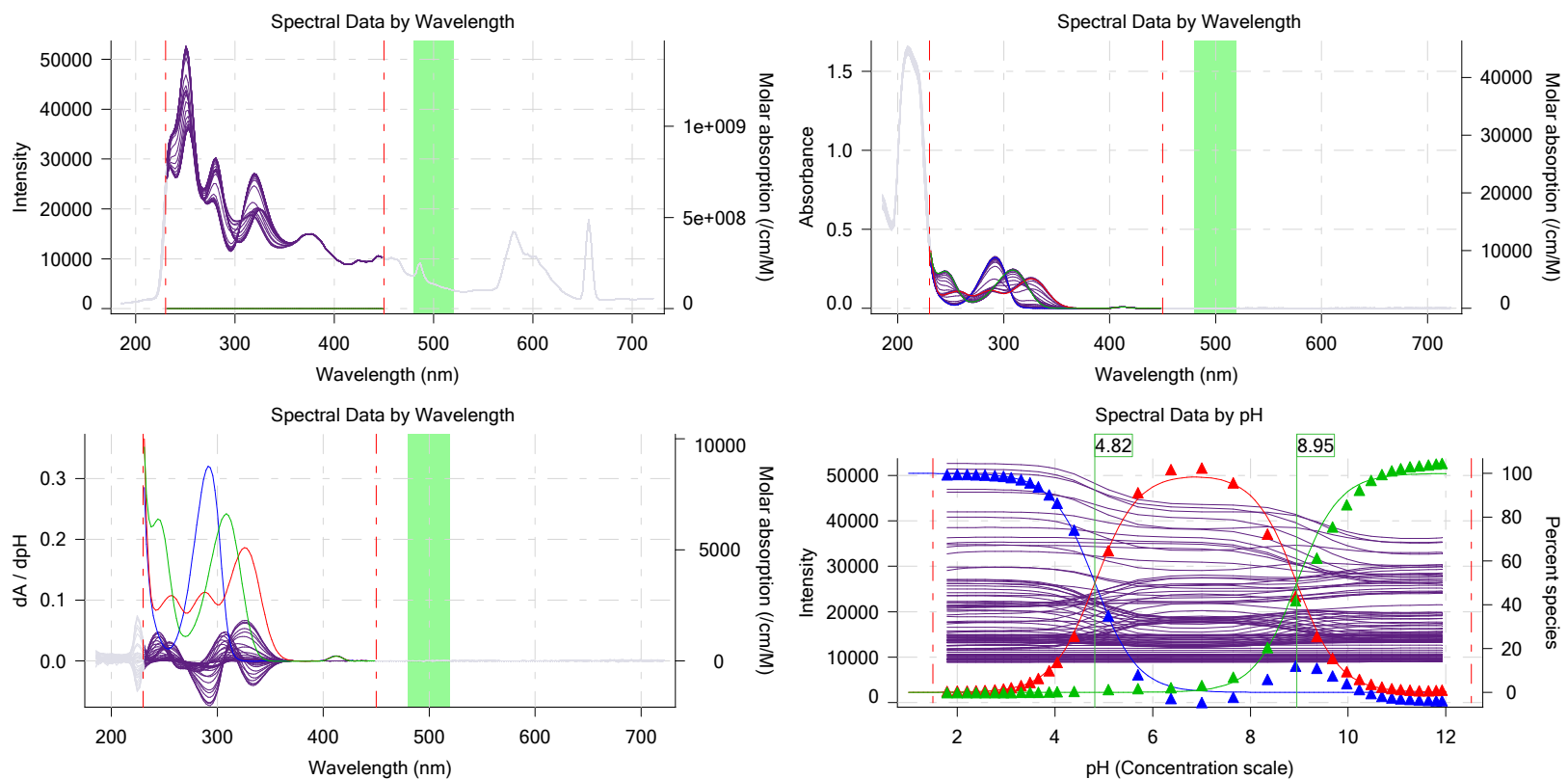
Errors **None**

Warnings **None**

Assay Settings

Setting	Value	Original Value	Date/Time changed	Imported from
Buffer in use	Yes			
Buffer type	Phosphate Buffer			
Assay Medium				
Volume of buffer introduced	0.025000 mL			
Add buffer manually	Manual			

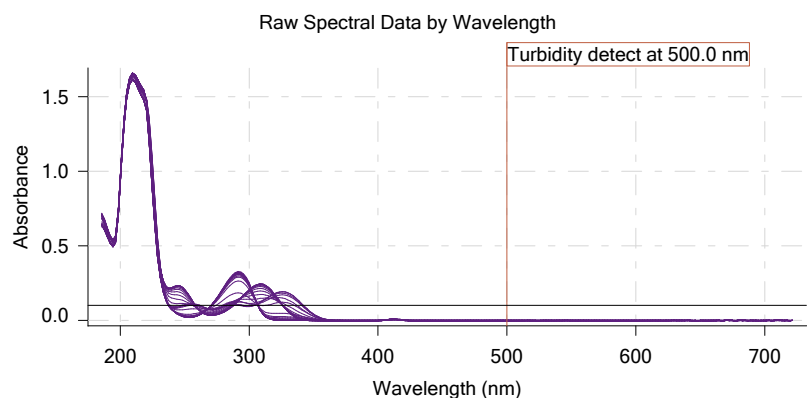
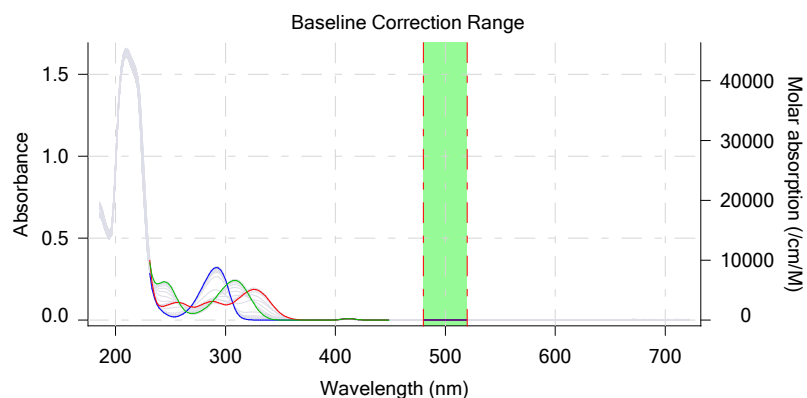
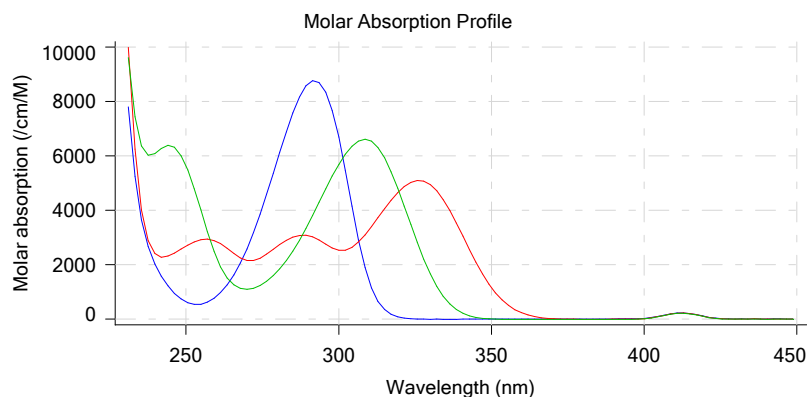
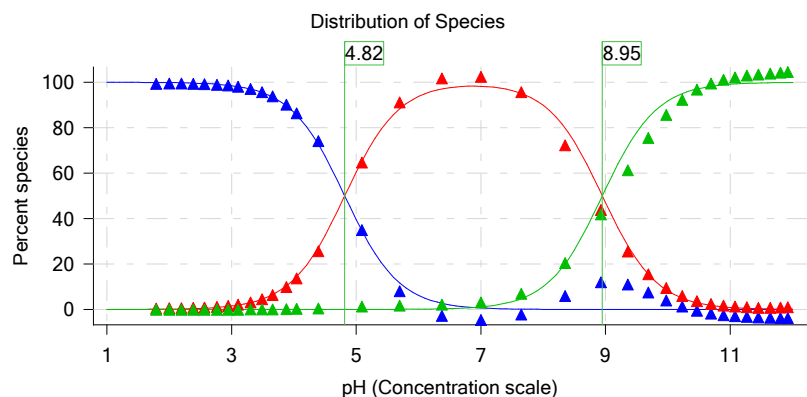
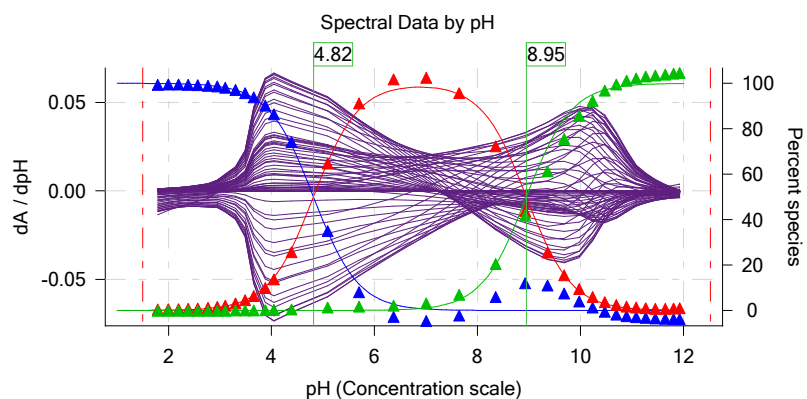
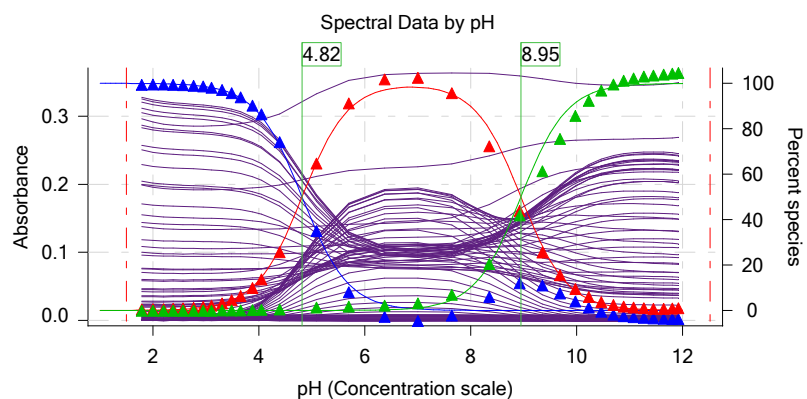
Graphs



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 Assay ID: **18E-22013**
 Filename: **C:\Sirius_T3\18E-22013_Pyridoxine HCl_UV-metric psKa_0417936-0002.t3r**

Experiment start time: **5/22/2018 2:50:50 PM**
 Analyst: **Dorothy Levorse**
 Instrument ID: **T311053**

Graphs (continued)



Assay Model

Settings

Settings	Value	Date/Time changed	Imported from
Sample name	Pyridoxine HCl	5/22/2018 9:07:27 AM	User entered value
Sample by	Volume		Default value
Sample volume	0.0020 mL	5/22/2018 9:07:27 AM	User entered value
Solvent	DMSO		Default value
Sample concentration	0.048630 M	5/22/2018 9:07:27 AM	User entered value
Solubility	Unknown		Default value
Molecular weight	205.64	5/22/2018 9:07:35 AM	User entered value
Individual pKa ionic environments	No		Default value
Number of pKas	2	5/22/2018 9:07:27 AM	User entered value
Sample is a	Ampholyte	5/22/2018 9:07:27 AM	User entered value
pKa 1	4.90	5/22/2018 9:07:27 AM	User entered value
Type	Base	5/22/2018 9:07:27 AM	User entered value
pKa 2	8.80	5/22/2018 9:07:27 AM	User entered value
Type	Acid	5/22/2018 9:07:27 AM	User entered value

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Experiment start time: **5/22/2018 2:50:50 PM**
 Analyst: **Dorothy Levorse**
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Assay Model (continued)

Settings	Value	Date/Time changed	Imported from
logp (XH2 +)	-10.00		Default value
logP (neutral XH)	-10.00	5/22/2018 9:07:27 AM	User entered value
logP (X -)	-10.00		Default value
Stoichiometry	1.00000		Default value
Aprotic counterion name	Chloride		From standards.xml file
Stoichiometry	1.00		From standards.xml file
Charge per counterion	-1		From standards.xml file

Events

Time	Event	Water	Acid	Base	Methanol	Buffer	pH	dpH/dt	pH R-square
3:47.5	Dark spectrum								
3:48.9	Reference spectrum								
4:16.6	Volume reset due to vial change								
5:00.8	Initial pH = 7.80								
6:14.0	Data point 4	0.34995 mL	0.07707 mL	0.00000 mL	1.15005 mL	0.02500 mL	1.958	-0.00675	0.36684
6:43.0	Data point 5	0.34995 mL	0.07707 mL	0.02787 mL	1.15005 mL	0.02500 mL	2.159	0.01880	0.73494
7:00.1	Data point 6	0.34995 mL	0.07707 mL	0.04473 mL	1.15005 mL	0.02500 mL	2.350	0.02418	0.81115
7:17.0	Data point 7	0.34995 mL	0.07707 mL	0.05543 mL	1.15005 mL	0.02500 mL	2.538	0.00766	0.48680
7:33.8	Data point 8	0.34995 mL	0.07707 mL	0.06251 mL	1.15005 mL	0.02500 mL	2.729	0.00940	0.69522
7:50.7	Data point 9	0.34995 mL	0.07707 mL	0.06698 mL	1.15005 mL	0.02500 mL	2.915	0.02148	0.94661
8:07.5	Data point 10	0.34995 mL	0.07707 mL	0.06987 mL	1.15005 mL	0.02500 mL	3.083	0.01310	0.92140
8:39.8	Data point 11	0.34995 mL	0.07707 mL	0.07180 mL	1.15005 mL	0.02500 mL	3.282	0.01748	0.95225
8:56.5	Data point 12	0.34995 mL	0.07707 mL	0.07305 mL	1.15005 mL	0.02500 mL	3.467	0.02450	0.97126
9:13.3	Data point 13	0.34995 mL	0.07707 mL	0.07385 mL	1.15005 mL	0.02500 mL	3.630	0.02600	0.98283
9:45.5	Data point 14	0.34995 mL	0.07707 mL	0.07462 mL	1.15005 mL	0.02500 mL	3.838	0.03686	0.95342
10:12.5	Data point 15	0.34995 mL	0.07707 mL	0.07502 mL	1.15005 mL	0.02500 mL	4.034	0.04323	0.92672
10:34.4	Data point 16	0.34995 mL	0.07707 mL	0.07538 mL	1.15005 mL	0.02500 mL	4.283	0.09629	0.99054
11:01.3	Data point 17	0.34995 mL	0.07707 mL	0.07563 mL	1.15005 mL	0.02500 mL	4.523	0.09939	0.99587
11:37.7	Data point 18	0.34995 mL	0.07707 mL	0.07589 mL	1.15005 mL	0.02500 mL	4.787	0.10033	0.99639
12:27.5	Data point 19	0.34995 mL	0.07707 mL	0.07627 mL	1.15005 mL	0.02500 mL	5.242	0.09340	0.95236
13:19.0	Data point 20	0.34995 mL	0.07707 mL	0.07639 mL	1.15005 mL	0.02500 mL	5.554	-0.06084	0.38499
13:54.7	Data point 21	0.34995 mL	0.07707 mL	0.07648 mL	1.15005 mL	0.02500 mL	6.113	0.12647	0.99662
15:11.6	Data point 22	0.34995 mL	0.07707 mL	0.07665 mL	1.15005 mL	0.02500 mL	6.733	0.09975	0.98674
16:16.1	Data point 23	0.34995 mL	0.07707 mL	0.07676 mL	1.15005 mL	0.02500 mL	7.168	0.09998	0.99022
17:12.6	Data point 24	0.34995 mL	0.07707 mL	0.07691 mL	1.15005 mL	0.02500 mL	7.505	0.10061	0.99032
17:59.0	Data point 25	0.34995 mL	0.07707 mL	0.07707 mL	1.15005 mL	0.02500 mL	7.810	0.09931	0.99002
18:44.0	Data point 26	0.34995 mL	0.07707 mL	0.07721 mL	1.15005 mL	0.02500 mL	8.082	0.09998	0.99187
19:28.4	Data point 27	0.34995 mL	0.07707 mL	0.07735 mL	1.15005 mL	0.02500 mL	8.371	0.09979	0.99259
20:08.2	Data point 28	0.34995 mL	0.07707 mL	0.07749 mL	1.15005 mL	0.02500 mL	8.718	0.09893	0.98482
20:58.7	Data point 29	0.34995 mL	0.07707 mL	0.07763 mL	1.15005 mL	0.02500 mL	9.090	0.09803	0.98111
21:40.6	Data point 30	0.34995 mL	0.07707 mL	0.07778 mL	1.15005 mL	0.02500 mL	9.429	0.09614	0.98682
22:21.0	Data point 31	0.34995 mL	0.07707 mL	0.07789 mL	1.15005 mL	0.02500 mL	9.673	0.10014	0.98157
22:54.0	Data point 32	0.34995 mL	0.07707 mL	0.07806 mL	1.15005 mL	0.02500 mL	9.914	0.09602	0.97678
23:22.1	Data point 33	0.34995 mL	0.07707 mL	0.07825 mL	1.15005 mL	0.02500 mL	10.124	0.07182	0.97071
23:43.8	Data point 34	0.34995 mL	0.07707 mL	0.07848 mL	1.15005 mL	0.02500 mL	10.357	0.04495	0.97842
24:00.5	Data point 35	0.34995 mL	0.07707 mL	0.07881 mL	1.15005 mL	0.02500 mL	10.577	0.01818	0.89241
24:17.0	Data point 36	0.34995 mL	0.07707 mL	0.07937 mL	1.15005 mL	0.02500 mL	10.811	0.00997	0.86656
24:49.1	Data point 37	0.34995 mL	0.07707 mL	0.08034 mL	1.15005 mL	0.02500 mL	11.010	0.00553	0.83760
25:05.8	Data point 38	0.34995 mL	0.07707 mL	0.08184 mL	1.15005 mL	0.02500 mL	11.201	0.00397	0.60445
25:22.5	Data point 39	0.34995 mL	0.07707 mL	0.08415 mL	1.15005 mL	0.02500 mL	11.387	0.00407	0.49542
25:39.2	Data point 40	0.34995 mL	0.07707 mL	0.08772 mL	1.15005 mL	0.02500 mL	11.562	0.00038	0.00606
25:56.0	Data point 41	0.34995 mL	0.07707 mL	0.09309 mL	1.15005 mL	0.02500 mL	11.754	-0.00112	0.03530
26:12.8	Data point 42	0.34995 mL	0.07707 mL	0.10153 mL	1.15005 mL	0.02500 mL	11.936	0.00241	0.18901
26:29.7	Data point 43	0.34995 mL	0.07707 mL	0.10823 mL	1.15005 mL	0.02500 mL	12.040	0.00108	0.05931
28:11.9	Reference spectrum								
29:16.3	Data point 45	0.50000 mL	0.19099 mL	0.10826 mL	1.15005 mL	0.02500 mL	1.975	-0.09545	0.97685



Assay Events

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Experiment start time: **5/22/2018 2:50:50 PM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

Events (continued)

Time	Event	Water	Acid	Base	Methanol	Buffer	pH	dpH/dt	pH R-squared	pH SD
29:46.1	Data point 46	0.50000 mL	0.19099 mL	0.13972 mL	1.15005 mL	0.02500 mL	2.171	0.00276	0.19246	0.00
30:03.2	Data point 47	0.50000 mL	0.19099 mL	0.15774 mL	1.15005 mL	0.02500 mL	2.361	0.01031	0.65796	0.00
30:20.1	Data point 48	0.50000 mL	0.19099 mL	0.16924 mL	1.15005 mL	0.02500 mL	2.562	0.00698	0.48137	0.00
30:36.9	Data point 49	0.50000 mL	0.19099 mL	0.17648 mL	1.15005 mL	0.02500 mL	2.749	0.00669	0.30673	0.00
30:53.8	Data point 50	0.50000 mL	0.19099 mL	0.18116 mL	1.15005 mL	0.02500 mL	2.955	0.01364	0.63909	0.00
31:10.5	Data point 51	0.50000 mL	0.19099 mL	0.18403 mL	1.15005 mL	0.02500 mL	3.145	0.02412	0.95389	0.00
31:27.3	Data point 52	0.50000 mL	0.19099 mL	0.18589 mL	1.15005 mL	0.02500 mL	3.323	0.03326	0.97931	0.00
31:44.1	Data point 53	0.50000 mL	0.19099 mL	0.18711 mL	1.15005 mL	0.02500 mL	3.506	0.03190	0.96348	0.00
32:00.8	Data point 54	0.50000 mL	0.19099 mL	0.18791 mL	1.15005 mL	0.02500 mL	3.707	0.03611	0.93670	0.00
32:17.4	Data point 55	0.50000 mL	0.19099 mL	0.18840 mL	1.15005 mL	0.02500 mL	3.886	0.03880	0.93500	0.00
32:34.1	Data point 56	0.50000 mL	0.19099 mL	0.18874 mL	1.15005 mL	0.02500 mL	4.052	0.06475	0.96862	0.00
32:55.8	Data point 57	0.50000 mL	0.19099 mL	0.18906 mL	1.15005 mL	0.02500 mL	4.321	0.09564	0.95362	0.00
33:18.2	Data point 58	0.50000 mL	0.19099 mL	0.18932 mL	1.15005 mL	0.02500 mL	4.695	0.09928	0.97259	0.00
33:53.7	Data point 59	0.50000 mL	0.19099 mL	0.18949 mL	1.15005 mL	0.02500 mL	4.975	0.09964	0.97032	0.00
34:22.9	Data point 60	0.50000 mL	0.19099 mL	0.18958 mL	1.15005 mL	0.02500 mL	5.260	0.09819	0.98682	0.00
34:59.2	Data point 61	0.50000 mL	0.19099 mL	0.18968 mL	1.15005 mL	0.02500 mL	5.649	0.09975	0.98785	0.00
35:47.6	Data point 62	0.50000 mL	0.19099 mL	0.18977 mL	1.15005 mL	0.02500 mL	6.090	0.09839	0.98247	0.00
36:34.4	Data point 63	0.50000 mL	0.19099 mL	0.18986 mL	1.15005 mL	0.02500 mL	6.486	0.09922	0.98693	0.00
37:20.3	Data point 64	0.50000 mL	0.19099 mL	0.18996 mL	1.15005 mL	0.02500 mL	6.799	0.10084	0.99471	0.00
38:01.1	Data point 65	0.50000 mL	0.19099 mL	0.19007 mL	1.15005 mL	0.02500 mL	7.115	0.09758	0.98427	0.00
38:43.0	Data point 66	0.50000 mL	0.19099 mL	0.19022 mL	1.15005 mL	0.02500 mL	7.428	0.09730	0.98030	0.00
39:18.4	Data point 67	0.50000 mL	0.19099 mL	0.19036 mL	1.15005 mL	0.02500 mL	7.727	0.09850	0.96000	0.00
39:57.4	Data point 68	0.50000 mL	0.19099 mL	0.19050 mL	1.15005 mL	0.02500 mL	8.004	0.09320	0.98471	0.00
40:38.8	Data point 69	0.50000 mL	0.19099 mL	0.19064 mL	1.15005 mL	0.02500 mL	8.324	0.09529	0.97727	0.00
41:17.8	Data point 70	0.50000 mL	0.19099 mL	0.19080 mL	1.15005 mL	0.02500 mL	8.758	0.09647	0.96427	0.00
41:57.2	Data point 71	0.50000 mL	0.19099 mL	0.19102 mL	1.15005 mL	0.02500 mL	9.264	0.09505	0.96325	0.00
42:30.6	Data point 72	0.50000 mL	0.19099 mL	0.19120 mL	1.15005 mL	0.02500 mL	9.535	0.09099	0.94785	0.00
43:00.5	Data point 73	0.50000 mL	0.19099 mL	0.19139 mL	1.15005 mL	0.02500 mL	9.758	0.09689	0.92452	0.00
43:22.7	Data point 74	0.50000 mL	0.19099 mL	0.19165 mL	1.15005 mL	0.02500 mL	10.010	0.05681	0.89973	0.00
43:44.5	Data point 75	0.50000 mL	0.19099 mL	0.19198 mL	1.15005 mL	0.02500 mL	10.212	0.02831	0.87650	0.00
44:16.5	Data point 76	0.50000 mL	0.19099 mL	0.19250 mL	1.15005 mL	0.02500 mL	10.409	0.03374	0.88383	0.00
44:48.6	Data point 77	0.50000 mL	0.19099 mL	0.19323 mL	1.15005 mL	0.02500 mL	10.606	0.01230	0.77321	0.00
45:15.6	Data point 78	0.50000 mL	0.19099 mL	0.19452 mL	1.15005 mL	0.02500 mL	10.818	0.00729	0.82566	0.00
45:42.6	Data point 79	0.50000 mL	0.19099 mL	0.19603 mL	1.15005 mL	0.02500 mL	11.010	0.00079	0.02605	0.00
45:59.4	Data point 80	0.50000 mL	0.19099 mL	0.19812 mL	1.15005 mL	0.02500 mL	11.218	0.00065	0.02108	0.00
46:16.2	Data point 81	0.50000 mL	0.19099 mL	0.20150 mL	1.15005 mL	0.02500 mL	11.399	-0.00077	0.03197	0.00
46:33.1	Data point 82	0.50000 mL	0.19099 mL	0.20666 mL	1.15005 mL	0.02500 mL	11.578	0.00407	0.41820	0.00
46:50.0	Data point 83	0.50000 mL	0.19099 mL	0.21449 mL	1.15005 mL	0.02500 mL	11.754	0.00080	0.04674	0.00
47:07.0	Data point 84	0.50000 mL	0.19099 mL	0.22636 mL	1.15005 mL	0.02500 mL	11.934	0.00185	0.12773	0.00
47:23.9	Data point 85	0.50000 mL	0.19099 mL	0.23594 mL	1.15005 mL	0.02500 mL	12.037	0.00236	0.21950	0.00
49:25.3	Reference spectrum									
50:33.5	Data point 87	0.83996 mL	0.32858 mL	0.23596 mL	1.15005 mL	0.02500 mL	1.997	-0.09665	0.98013	0.00
51:17.1	Data point 88	0.83996 mL	0.32858 mL	0.27225 mL	1.15005 mL	0.02500 mL	2.198	-0.00138	0.06588	0.00
51:44.6	Data point 89	0.83996 mL	0.32858 mL	0.29231 mL	1.15005 mL	0.02500 mL	2.393	0.00349	0.09831	0.00
52:01.6	Data point 90	0.83996 mL	0.32858 mL	0.30456 mL	1.15005 mL	0.02500 mL	2.576	-0.02010	0.83036	0.00
52:18.6	Data point 91	0.83996 mL	0.32858 mL	0.31268 mL	1.15005 mL	0.02500 mL	2.756	-0.02760	0.83347	0.00
52:35.4	Data point 92	0.83996 mL	0.32858 mL	0.31801 mL	1.15005 mL	0.02500 mL	2.954	0.02357	0.77780	0.00
52:52.2	Data point 93	0.83996 mL	0.32858 mL	0.32133 mL	1.15005 mL	0.02500 mL	3.132	0.00080	0.02816	0.00
53:08.9	Data point 94	0.83996 mL	0.32858 mL	0.32356 mL	1.15005 mL	0.02500 mL	3.288	0.01078	0.80012	0.00
53:35.9	Data point 95	0.83996 mL	0.32858 mL	0.32502 mL	1.15005 mL	0.02500 mL	3.486	0.02594	0.98050	0.00
53:52.6	Data point 96	0.83996 mL	0.32858 mL	0.32599 mL	1.15005 mL	0.02500 mL	3.669	0.02302	0.90868	0.00
54:09.2	Data point 97	0.83996 mL	0.32858 mL	0.32662 mL	1.15005 mL	0.02500 mL	3.835	0.03133	0.98006	0.00
54:31.0	Data point 98	0.83996 mL	0.32858 mL	0.32712 mL	1.15005 mL	0.02500 mL	4.053	0.09511	0.99496	0.00
54:47.7	Data point 99	0.83996 mL	0.32858 mL	0.32738 mL	1.15005 mL	0.02500 mL	4.221	0.09919	0.98807	0.00
55:32.0	Data point 100	0.83996 mL	0.32858 mL	0.32773 mL	1.15005 mL	0.02500 mL	4.563	0.09992	0.98994	0.00
56:15.6	Data point 101	0.83996 mL	0.32858 mL	0.32815 mL	1.15005 mL	0.02500 mL	5.255	0.09800	0.98954	0.00
57:07.0	Data point 102	0.83996 mL	0.32858 mL	0.32836 mL	1.15005 mL	0.02500 mL	5.858	0.09893	0.99333	0.00

Sample name: **Pyridoxine HCl**
 Assay name: **UV-metric psKa**
 Assay ID: **18E-22013**
 Filename: **C:\Sirius_T3\18E-22013_Pyridoxine HCl_UV-metric psKa_0417936-0002.t3r**

Experiment start time: **5/22/2018 2:50:50 PM**
 Analyst: **Dorothy Levorse**
 Instrument ID: **T311053**

Events (continued)

Time	Event	Water	Acid	Base	Methanol	Buffer	pH	dpH/dt	pH R-squared	pH SD
58:12.4	Data point 103	0.83996 mL	0.32858 mL	0.32867 mL	1.15005 mL	0.02500 mL	6.528	0.08309	0.76810	0.0046
58:42.8	Data point 104	0.83996 mL	0.32858 mL	0.32898 mL	1.15005 mL	0.02500 mL	7.152	0.06530	0.59596	0.0041
59:16.2	Data point 105	0.83996 mL	0.32858 mL	0.32935 mL	1.15005 mL	0.02500 mL	7.790	0.09756	0.98263	0.0048
59:56.6	Data point 106	0.83996 mL	0.32858 mL	0.32966 mL	1.15005 mL	0.02500 mL	8.488	0.09740	0.97254	0.0048
1:00:49.8	Data point 107	0.83996 mL	0.32858 mL	0.32999 mL	1.15005 mL	0.02500 mL	9.057	0.09636	0.98563	0.0047
1:01:37.7	Data point 108	0.83996 mL	0.32858 mL	0.33034 mL	1.15005 mL	0.02500 mL	9.486	0.09860	0.97841	0.0049
1:02:10.7	Data point 109	0.83996 mL	0.32858 mL	0.33100 mL	1.15005 mL	0.02500 mL	9.811	0.10007	0.99467	0.0049
1:02:38.7	Data point 110	0.83996 mL	0.32858 mL	0.33175 mL	1.15005 mL	0.02500 mL	10.098	0.06725	0.93744	0.0034
1:03:00.6	Data point 111	0.83996 mL	0.32858 mL	0.33248 mL	1.15005 mL	0.02500 mL	10.353	0.02323	0.91021	0.0012
1:03:32.7	Data point 112	0.83996 mL	0.32858 mL	0.33354 mL	1.15005 mL	0.02500 mL	10.588	0.01887	0.91930	0.0009
1:04:04.9	Data point 113	0.83996 mL	0.32858 mL	0.33481 mL	1.15005 mL	0.02500 mL	10.811	0.00541	0.59517	0.0003
1:04:21.6	Data point 114	0.83996 mL	0.32858 mL	0.33683 mL	1.15005 mL	0.02500 mL	11.007	-0.01169	0.83876	0.0006
1:04:38.2	Data point 115	0.83996 mL	0.32858 mL	0.34001 mL	1.15005 mL	0.02500 mL	11.191	-0.01953	0.88709	0.0010
1:04:55.1	Data point 116	0.83996 mL	0.32858 mL	0.34485 mL	1.15005 mL	0.02500 mL	11.379	-0.01258	0.84686	0.0006
1:05:12.0	Data point 117	0.83996 mL	0.32858 mL	0.35237 mL	1.15005 mL	0.02500 mL	11.562	-0.00673	0.47279	0.0004
1:05:29.0	Data point 118	0.83996 mL	0.32858 mL	0.36395 mL	1.15005 mL	0.02500 mL	11.749	-0.01133	0.90688	0.0005
1:05:46.1	Data point 119	0.83996 mL	0.32858 mL	0.38208 mL	1.15005 mL	0.02500 mL	11.906	-0.00971	0.51929	0.0006
1:06:08.4	Data point 120	0.83996 mL	0.32858 mL	0.39866 mL	1.15005 mL	0.02500 mL	12.023	-0.00268	0.19720	0.0003
1:08:13.7	Assay volumes	1.08996 mL	0.49266 mL	0.39866 mL	1.15005 mL	0.02500 mL				

Assay Settings

Setting	Value	Original Value	Date/Time changed	Imported from
General Settings				
Analyst name	Dorothy Levorse			
Separate reference vial	Yes			
Standard Experiment Settings				
Number of titrations	3			
Minimum pH	2.000			
Maximum pH	12.000			
pH step between points of	0.200			
Minimum titrant addition	0.00002 mL			
Maximum titrant addition	0.10000 mL			
Argon flow rate	100%			
Start titration using	Cautious pH adjust			
Advanced General Settings				
Detect turbidity using	Spectrometer			
Monitor at a wavelength of	500.0 nm			
Absorbance threshold of	0.100			
Collect turbidity sensor data	No			
Stir after titrant addition for	5 seconds			
For titrant addition, stir at	15%			
Titration Pre-Dose				
Titration pre-dose	None			
Assay Medium				
Cosolvent in use	Yes			
Cosolvent type	Methanol			
Cosolvent volume	1.15 mL			
Cosolvent added	Automatic			
ISA water volume	0.35 mL			
Water added	Automatic			
After water addition, stir for	5 seconds			
At a speed of	15%			
Buffer in use	Yes			
Buffer type	Phosphate Buffer			
Volume of buffer introduced	0.025000 mL			
Add buffer manually	Manual			
After medium addition, stir for	5 seconds			

Sample name: **Pyridoxine HCl**
 Assay name: **UV-metric psKa**
 Assay ID: **18E-22013**
 Filename: **C:\Sirius_T3\18E-22013_Pyridoxine HCl_UV-metric psKa_0417936-0002.t3r**

Experiment start time: **5/22/2018 2:50:50 PM**
 Analyst: **Dorothy Levorse**
 Instrument ID: **T311053**

Assay Settings (continued)

Setting	Value	Original Value	Date/Time changed	Imported from
Sample Sonication				
Sonicate	No			
Sample Dissolution				
Perform a dissolution stage	No			
Carbonate purge				
Perform a carbonate purge	No			
Temperature Control				
Wait for temperature	Yes			
Required start temperature	25.0°C			
Acceptable deviation	0.5°C			
Time to wait	60 seconds			
Stir speed of	15%			
Titration 1				
Titrate from	Low to high pH			
Adjust to start pH	Yes			
After pH adjust stir for	10 seconds			
Titration 2				
Titrate from	Low to high pH			
Additional cosolvent volume	0.00 mL			
Add additional water	0.15 mL			
Additional water added	Automatic			
After pH adjust stir for	10 seconds			
Titration 3				
Titrate from	Low to high pH			
Additional cosolvent volume	0.00 mL			
Add additional water	0.34 mL			
Additional water added	Automatic			
After pH adjust stir for	10 seconds			
Data Point Stability				
Stir during data point collection	Yes			
For point collection, stir at	15%			
Delay before data point collection	0 seconds			
Number of points to average	20 points			
Time interval between points	0.50 seconds			
Required maximum standard deviation	0.00500 dpH/dt			
Stability timeout after	60 seconds			
Experiment cleanup				
Adjust pH to cleanup	To start pH			
And then stir for	60 seconds			
For cleaning, stir at	20%			
Then add water volume	0.25 mL			
And then stir for	30 seconds			

Calibration Settings

Setting	Value	Date/Time changed	Imported from
Four-Plus alpha	0.144	5/22/2018 2:50:50 PM	C:\Sirius_T3\18E-22009_Blank standardisation.t3r
Four-Plus S	0.9948	5/22/2018 2:50:50 PM	C:\Sirius_T3\18E-22009_Blank standardisation.t3r
Four-Plus jH	1.0	5/22/2018 2:50:50 PM	C:\Sirius_T3\18E-22009_Blank standardisation.t3r
Four-Plus jOH	-0.8	5/22/2018 2:50:50 PM	C:\Sirius_T3\18E-22009_Blank standardisation.t3r
Base concentration factor	1.012	5/22/2018 2:50:50 PM	C:\Sirius_T3\KOH18D10.t3r
Acid concentration factor	0.998	5/22/2018 2:50:50 PM	C:\Sirius_T3\18E-22009_Blank standardisation.t3r

Instrument Settings

Setting	Value	Batch Id	Install date
Instrument owner	Merck		
Instrument ID	T311053		

Sample name: **Pyridoxine HCl**
 Assay name: **UV-metric psKa**
 Assay ID: **18E-22013**
 Filename: **C:\Sirius_T3\18E-22013_Pyridoxine HCl_UV-metric psKa_0417936-0002.t3r**

Experiment start time: **5/22/2018 2:50:50 PM**
 Analyst: **Dorothy Levorse**
 Instrument ID: **T311053**

Instrument Settings (continued)

Setting	Value	Batch Id	Install date
Instrument type	T3 Simulator		
Software version	1.1.3.0		
Dispenser module		T3DM1100253	3/31/2009 6:24:52 AM
Dispenser 0	Water		3/31/2009 6:25:05 AM
Syringe volume	2.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Water (0.15 M KCl)	2-6-18	5/15/2018 2:12:22 PM
Dispenser 2	Acid		3/31/2009 6:25:11 AM
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Acid (0.5 M HCl)	3-22-18	5/15/2018 2:12:48 PM
Dispenser 1	Base		3/31/2009 6:25:21 AM
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Base (0.5 M KOH)	3-22-18	5/15/2018 2:12:34 PM
Dispenser 5	Cosolvent		3/31/2009 6:26:24 AM
Syringe volume	2.5 mL		
Firmware version	1.2.1(r2)		
Distribution valve 5	Distribution Valve		3/31/2009 6:28:19 AM
Firmware version	1.1.3		
Port A	Methanol (80%, 0.15 M KCl)	2-8-18	5/15/2018 2:14:14 PM
Port B	Cyclohexane		4/10/2018 8:40:51 AM
Port C	MeCN (50%, 0.15 M KCl)	4-16-18	5/15/2018 2:14:20 PM
Dispenser 3	Buffer		8/3/2010 6:05:16 AM
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Dodecane	1-31-2018	5/15/2018 2:12:54 PM
Dispenser 6	Octanol		10/22/2010 11:52:43 AM
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Octanol	1-31-2018	4/9/2018 9:14:11 AM
Titration		T3TM1100153	3/31/2009 6:24:17 AM
Horizontal axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Vertical axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Chassis I/O firmware version	1.11 AI1DI0DO4 Norgren I/O		
Probe I/O firmware version	1.1.1		
Electrode	T3 Electrode	T3E0769	8/15/2017 10:21:54 AM
E0 calibration	-12.44 mV		5/22/2018 2:51:14 PM
Filling solution	3M KCl	KCL095	5/21/2018 8:57:01 AM
Liquids			
Wash 1	50% IPA:50% Water		5/22/2018 8:38:15 AM
Wash 2	0.5% Triton X-100 in H2O		5/22/2018 8:38:18 AM
Buffer position 1	pH7 Wash		5/22/2018 8:38:22 AM
Buffer position 2	pH 7		5/22/2018 8:38:25 AM
Storage position			5/22/2018 8:38:32 AM
Wash water	3.2e+003 mL	5-15-18	5/15/2018 2:11:48 PM
Waste	7.3e+003 mL		3/19/2018 10:48:12 AM
Temperature controller			8/5/2010 7:35:13 AM
Turbidity detector			3/31/2009 6:24:45 AM
Spectrometer		072390	11/23/2010 12:22:28 PM
Dip probe		11086	
Wavelength coefficient A0	185.563		
Wavelength coefficient A1	2.17439		
Wavelength coefficient A2	-0.000285622		
Total lamp lit time	897:26:49		11/23/2010 12:22:28 PM
Calibrated on	5/21/2018 2:44:22 PM		
Integration time	19		
Scans averaged	10		



Assay Settings

Sample name: **Pyridoxine HCl**
Assay name: **UV-metric psKa**
Assay ID: **18E-22013**
Filename: **C:\Sirius_T3\18E-22013_Pyridoxine HCl_UV-metric psKa_0417936-0002.t3r**

Experiment start time: **5/22/2018 2:50:50 PM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

Instrument Settings (continued)

Setting	Value	Batch Id	Install date
Autoloader		T3AL1100237	11/10/2015 10:34:13 AM
Left-right axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Front-back axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Vertical axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Chassis I/O firmware version	1.11 AI1DI0DO4 Norgren I/O		
Configuration			
Alternate titration position	Titration position		
Alternate reference position	Reference position		
Maximum standard vial volume	3.50 mL		
Maximum alternate vial volume	25.00 mL		
Automatic action idle period	5 minute(s)		
Titrant tube volume	1.3 mL		
Syringe flush count	3.50		
Flowing wash pump volume	20.0 mL		
Flowing wash stir duration	5 s		
Flowing wash stir speed	30%		
Solvent wash stir duration	5 s		
Solvent wash stir speed	30%		
Surfactant wash stir duration	5 s		
Surfactant wash stir speed	30%		
E0 calibration minimum number of points	10		
E0 calibration maximum standard deviation	0.01500		
E0 calibration timeout period	60 s		
E0 calibration stir duration	5 s		
E0 calibration preparation stir speed	30%		
E0 calibration buffer wash stir duration	5 s		
E0 calibration buffer wash stir speed	30%		
E0 calibration reading stir speed	0%		
Spectrometer calibration stir duration	5 s		
Spectrometer calibration stir speed	30%		
Spectrometer calibration wash pump volume	20.0 mL		
Spectrometer calibration wash stir duration	5 s		
Spectrometer calibration wash stir speed	30%		
Overhead dispense height	10000		

Refinement Settings

Setting	Value	Default value
Turbidity detection method	Spectrometer	Spectrometer
Turbidity wavelength to assess	500.0 nm	500.0 nm
Turbidity maximum absorbance	0.100	0.100
Turbidity probe threshold	50.00	50.00
Exclude turbid points	Yes	Yes
Low intensity warning threshold	100	100
Minimum absorbance change threshold	0.100	0.100
Eigenvector autocorrelation threshold	0.80	0.80
Maximum RMSD severe warning	0.250	0.250
Maximum RMSD warning	0.050	0.050

Tray Information

Title
Location B5